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(54) **Novel chemokine produced by hematopoietic cells and DNA encoding said chemokine**

Neues Chemokine von hematopoietischen Zellen produziert und die dafür kodierende DNA

Nouvelle chemokine produit par des cellules hématopoiétiques et ADN codant pour celle-ci

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EP-A- 0 244 042 **WO-A-92/18521**
DE-A- 3 901 681

- **NUCL. ACIDS RES., vol.19, no.25, December 1991, IRL PRESS, OXFORD, ENGLAND; pages 7097 - 7104 E. HARA ET AL. 'Substractive cDNA cloning using oligo(dT)30-latex and PCR: isolation of cDNA clones specific to undifferentiated human embryonal carcinoma cells'**
- **SCIENCE, vol.261, 30 July 1993, AAAS, WASHINGTON, DC, US; pages 600 - 603 K. TASHIRO ET AL. 'Signal sequence trap: A cloning strategy for secreted proteins and type I membrane proteins'**
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EP 0 607 054 B1

Description

[0001] This invention relates to a novel polypeptide and a DNA coding for the same. More particularly, it relates to a novel polypeptide produced by a specific stroma cell line and a DNA coding for said polypeptide.

[0002] In order to obtain specific polypeptides (for example, proliferation and/or differentiation factors) or a DNA coding for the same, there have been generally employed methods comprising confirming the target biological activity in a tissue or a cell culture medium and then cloning of a gene through the isolation and purification of a polypeptide and further methods comprising expression-cloning of a gene with the guidance of the biological activity.

[0003] However, it is frequently observed that a gene, which has been cloned with the guidance of a certain activity, codes for a known polypeptide since many physiologically active polypeptides occurring *in vivo* have various biological activities. Further, most intravital physiologically active polypeptides are secreted only in a trace amount, which makes the isolation and purification thereof and the confirmation of biological activity difficult.

[0004] Recent rapid developments in techniques for constructing cDNAs and sequencing techniques have made it possible to quickly sequence a large amount of cDNAs. By utilising these techniques, a process, which comprises constructing cDNA libraries from various cells and tissues, cloning cDNAs at random, identifying the nucleotide sequences thereof, expressing the corresponding polypeptide and then analyzing its physiological functions, is now in use. Although this process is advantageous in that a gene can be cloned and information on its nucleotide sequence can be obtained without any biochemical or genetic analysis, the target gene can often only be identified by chance.

[0005] The present inventors have studied the cloning of genes for proliferation and differentiation factors in hematopoietic and immune systems. They have paid attention to the fact that most secretory proteins such as proliferation and/or differentiation factors (for example various cytokines) and membrane proteins such as receptors thereof (hereinafter these proteins will be referred to generally as secretory proteins and the like) have sequences called signal peptides in the N-termini. Extensive studies have been conducted to provide a process for efficiently and selectively cloning genes coding for signal peptides. As a result, a process has now been devised whereby an N-terminal fragment can be efficiently amplified and the existence of a signal peptide can be easily examined.

[0006] In accordance with this process, cDNAs with a high probability of containing a signal peptide are ligated at both their ends to linkers containing restriction enzyme sites which are different from each other. These fragments are rapidly produced in a large amount by the polymerase chain reaction (PCR) method so as to elevate the content of the fragments with a high probability of containing a signal peptide. Next, the above-mentioned fragment is introduced into an expression vector containing DNA coding for a known secretory protein or the like but lacking DNA encoding the corresponding signal peptide. Many secretory proteins and the like are secreted or expressed on cell membrane even if the signal peptide has been substituted by a signal peptide of another protein. Therefore, if the known secretory protein or the like is expressed on cell membrane or outside the cells, this confirms that a cDNA fragment corresponding to a signal peptide has been introduced into the expression vector. Thus the inventors have devised a convenient method for detection of the insertion of cDNA encoding a signal peptide.

[0007] The polymerase chain reaction is known as a method for amplifying specific DNA fragments in a large amount. It is also known that many secretory proteins and the like can be expressed even if the signal peptide thereof is substituted by that of another secretory protein or the like. However, it is believed that there has been no suggestion that these techniques be used as the inventors have been done to provide a process for selectively cloning a signal peptide.

[0008] It is known that hematopoietic cells secrete various proliferation and/or differentiation factors exemplified by interleukin. Using the process they have devised, the inventors have sought a novel factor (polypeptide) produced by hematopoietic cells. As a result, a novel polypeptide and DNA coding for the same have been identified.

[0009] Computer searches to identify polypeptides having sequences identical or highly homologous with that of the polypeptide of the present invention and the DNAs coding for the same have not identified any such sequences. Thus, the polypeptide of the present invention and the DNA coding for the same are believed to be novel. Further, homology analysis has revealed that the polypeptide of the present invention is a member of the chemokine family as it has a pattern of Cys-X-Cys (X is optional amino acid).

[0010] Accordingly, the present invention provides a polypeptide in substantially purified form having the amino acid shown in SEQ ID NO:1, the amino acid sequence of residues 1 to 70 shown in SEQ ID NO:1 or an amino acid sequence having at least 90% homology with the amino acid sequence shown in SEQ ID NO:1 over its entire length or with the amino acid sequence of residues 1 to 70 shown in SEQ ID NO:1. DNA encoding such a polypeptide is also provided. The polypeptide having the sequence shown in SEQ ID NO:1 has been identified using the process devised by the inventors.

[0011] The invention further provides:

- a replication and an expression vector comprising DNA of the invention;
- a host cell transformed or transfected with a vector of the invention;
- a method of producing a polypeptide which method comprises culturing host cells of the invention under conditions

effective to express a polypeptide of the invention;

- a monoclonal or polyclonal antibody to a polypeptide of the invention; and
- a pharmaceutical composition containing a polypeptide or antibody of the invention in association with a pharmaceutically acceptable diluent or carrier.

Fig. 1 is a conceptional view of the process for constructing a cDNA library according to the process devised by the inventors.
Fig. 2 is a flow chart for the construction of a plasmid vector pcDL-SRα.
Fig. 3 is a conceptional view of the process for constructing an EcoRI-SacI fragment of hG-CSF.
Fig. 4 is a conceptional view of the process for constructing an SacI-KpnI fragment of hTac cDNA.
Fig. 5 is a flow chart for the construction of pSGT and pSRT.
Fig. 6 is a conceptional view of the process for constructing an EcoRI-SacI fragment of hRARα.
Fig. 7 is an FACS histogram showing the expression of a fusion protein hG-CSF-hTac on membrane.
Fig. 8 is an FACS histogram showing the expression of a fusion protein hRAR-hTac on membrane.
Fig. 9 is a conceptional view of the first half of the process for constructing the cDNA library of the Example.
Fig. 10 is a conceptional view of the second half of the process or constructing the cDNA library of the Example.
Fig. 11 is a hydrophobicity profile of (a part of) the polypeptide according to the present invention.

[0012] The present invention thus relates to a novel polypeptide which has been identified using the process devised by the present inventors to construct a cDNA library. In particular, it relates to:

(1) a polypeptide consisting of an amino acid sequence represented by SEQ ID NO. 1;
(2) a DNA coding for the polypeptide (1) ;
(3) a DNA having a nucleotide sequence represented by SEQ ID No. 4; and
(4) a DNA having a nucleotide sequence represented by SEQ ID NO. 3.

[0013] A polypeptide of Seq. ID No. 1 in substantially purified form will generally comprise the polypeptide in a preparation in which more than 90%, eg. 95%, 98% or 99% of the polypeptide in the preparation is that of the Seq. ID No. 1.

[0014] A polypeptide homologue of the Seq. ID No. 1 will be at least 90% and more preferably at least 95% homologous to the polypeptide of Seq. ID No. 1 over a region of at least 30, for instance 40, 60 or 100 more contiguous amino acids. Such polypeptide homologues will be referred to below as a polypeptide according to the invention.

[0015] Generally, fragments of Seq. ID No. 1 or its homologues will be at least 30, for example 40, 50 or 60 amino acids in length, and are also encompassed by the term "a polypeptide according to the invention" as used herein. Particular fragments of the polypeptides of the invention are fragments which include amino acid residues numbered 1-70 in Seq ID No. 1 or a homologue thereof.

[0016] A DNA capable of selectively hybridizing to the DNA of Seq. ID No. 3 or 4 will be generally at least 90% and more preferably at least 95% homologous to the DNA of Seq. ID No. 3 or 4 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 or more contiguous nucleotides. Such DNA will be encompassed by the term "DNA according to the invention".

[0017] Particular DNA capable of selectively hybridising to the DNA of SEQ ID No. 3 or 4 is the nucleotide residues numbered 139-348 in SEQ ID No. 1 or a fragment thereof.

[0018] DNA according to the invention may be used to produce a primer, eg. a PCR primer, a probe eg. labelled by conventional means using radioactive or nonradioactive labels, or the DNA may be cloned into a vector. Such primers, probes and other fragments of the DNA of Seq. ID No. 3 or 4 will be at least 15, preferably at least 20, for example 25, 30 or 40 nucleotides in length.

[0019] DNA according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art.

[0020] A further embodiment of the invention provides replication and expression vectors comprising DNA according to the invention. The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said DNA and optionally a regulator of the promotor. The vector may contain one or more selectable marker genes, for example an ampicillin resistance gene. The vector may be used in vitro, for example for the production of RNA corresponding to the DNA, or used to transform a host cell.

[0021] A further embodiment of the invention provides host cells transformed or transfected with the vectors for the replication and expression of DNA according to the invention, including the DNA Seq. ID No.3 or 4 or the open reading frame thereof. The cells will be chosen to be compatible with the vector and may for example be bacterial, yeast, insect or mammalian.

[0022] DNA according to the invention may also be inserted into the vectors described above in an antisense orientation in order to provide for the production of antisense RNA (DNA). Antisense RNA (DNA) may also be produced by

synthetic means. Such antisense RNA (DNA) may be used in a method of controlling the levels of a polypeptide of the invention in a cell.

[0023] A further embodiment of the invention provides a method of producing a polypeptide which comprises culturing host cells of the present invention under conditions effective to express a polypeptide of the invention. Preferably, in addition, such a method is carried out under conditions in which the polypeptide of the invention is expressed and then secreted from the host cells.

[0024] The invention also provides monoclonal or polyclonal antibodies to a polypeptide according to the invention. The invention further provides a process for the production of monoclonal or polyclonal antibodies to the polypeptides of the invention. Monoclonal antibodies may be prepared by conventional hybridoma technology using a polypeptide of the invention or a fragment thereof, as an immunogen. Polyclonal antibodies may also be prepared by conventional means which comprise inoculating a host animal, for example a rat or a rabbit, with a polypeptide of the invention and recovering immune serum.

[0025] The present invention also provides pharmaceutical compositions containing a polypeptide of the invention, or an antibody thereof, in association with a pharmaceutically acceptable diluent or carrier.

[0026] The invention also provides a polypeptide according to the invention or an antibody for use in a method of therapy or diagnosis on a human or animal body.

[0027] The polypeptides of the present invention include not only those having the amino acid sequence represented by the SEQ ID No. 1 but also those with partial deletion thereof (for example, a polypeptide consisting of the mature protein part alone, or consisting of a part of the mature protein essentially required for the expression of the biological activity), those with partial replacement by other amino acid(s) (for example, a polypeptide some of amino acids are replaced by those having similar properties) and those with partial addition or insertion of amino acid(s).

[0028] It is well known that there are up to six different codons which may code for a single amino acid (for example, one type of codon for Met while six types of codon for Leu). Accordingly, the nucleotide sequence of the DNA can be changed without altering the amino acid sequence of the polypeptide.

[0029] The DNA as specified in (2) includes all nucleotide sequences coding for the polypeptide represented by Seq. ID NO. 1. Changes in the nucleotide sequence sometimes bring about an increase in the polypeptide productivity.

[0030] The DNA as specified in (3) is an embodiment of the DNA as specified in (2) and represents a natural sequence.

[0031] The DNA as specified in (4) represents a sequence wherein a natural non-translational region is added to the DNA as specified in (3).

[0032] A signal peptide is a highly hydrophobic region located immediately downstream of the translation initiation amino acid Met. It is assumed that the signal peptide in the polypeptide of the present invention resides in a region ranging from Met at the 1-position to Ser at the 19-position in the amino acid sequence represented by Seq. ID No. 1. The region essentially responsible for the expression of the biological activity corresponds to the part of the amino acid sequence of the Seq. ID No. 1 lacking of the signal peptide, i.e., the mature protein part. Thus the signal peptide never relates to the activity.

[0033] Once the nucleotide sequences represented by Seq. ID No. 3 and No. 4 are determined, the DNA of the present invention can be chemically synthesized. Alternatively, the DNA of the present invention can be obtained by chemically synthesizing fragments of said nucleotide sequence and hybridizing with the use of the fragments as a probe. Further, the target DNA can be produced in a desired amount by introducing a vector DNA containing said DNA into an appropriate host and then incubating the host.

[0034] Examples of methods for obtaining the polypeptide of the present invention include:

(1) isolation and purification from vital tissues or cultured cells;
(2) chemical synthesis of peptides; and
(3) production with the use of gene recombination techniques. From an industrial viewpoint, the method described in (3) is preferable.

[0035] Examples of the expression system (host-vector system) for producing the polypeptide by using gene recombination techniques include those of bacteria, yeasts, insect cells and mammalian cells.

[0036] In order to express in *E. coli*, for example, an initiator codon (ATG) is added to the 5'-end of the DNA coding for the mature protein region. The DNA thus obtained is then ligated to the downstream of an appropriate promoter (for example, trp promoter, lac promoter, λp_L promoter, T7 promoter, etc.) and inserted into a vector capable of functioning in *E. coli* (for example, pBR322, pUC18, pUC19, etc.), thus constructing an expression vector. Next, an *E. coil* strain (for example, *E. coli* DH1, *E. coli* JM109, *E. coli* HB101, etc.) transformed with this expression vector is incubated in an appropriate medium. Thus the target polypeptide can be obtained from the incubated cells. Alternately, a bacterial signal peptide (for example, a signal peptide of pelB) may be used and thus the polypeptide can be secreted into the periplasm. Furthermore, a fusion protein together with other polypeptide can be produced.

[0037] Expression in mammalian cells can be effected, for example, in the following manner. Namely, a DNA coding for the nucleotide sequence represented by Seq. ID No. 3 is inserted into the downstream of an appropriate promoter (for example, SV40 promoter, LTR promoter, metallothionein promoter, etc.) in an appropriate vector (for example, retrovirus vector, papilloma virus vector, vaccinia virus vector, SV40-series vector, etc.), thus constructing an expression vector. Next, appropriate mammalian cells (for example, monkey COS-7 cells, Chinese hamster CHO cells, mouse L cells, etc.) are transformed with the expression vector obtained above and the transformant is incubated in an appropriate medium. Thus the target polypeptide can be secreted into the culture medium. The polypeptide thus obtained can be isolated and purified by conventional biochemical methods.

[0038] The novel polypeptide which is the subject of the present invention is produced and secreted from a stroma cell line. Therefore, the polypeptide has biological activities relating to the survival and proliferation of hematopoietic stem cells and the proliferation and differentiation of B cells and myeloid cells, and chemoattractant activity of neurophil. Accordingly, the polypeptide of the present invention per se is usable as an agent for preventing or treating, for example, anemia or leukopenia, infections, etc.

[0039] In addition, the above-mentioned polypeptide existing in vivo can be assayed by using a polyclonal antibody or a monoclonal antibody for said polypeptide, which is applicable to studies on the relationship between said polypeptide and diseases or to the diagnosis of diseases and the like. The polyclonal antibody and the monoclonal antibody can be prepared by a conventional method with the use of said polypeptide or a fragment thereof as an antigen.

[0040] The DNA according to the present invention serves as an important and essential template in the production of the polypeptide of the present invention which is expected to be highly useful. Further, the DNA of the present invention is applicable to the diagnosis and treatment of hereditary diseases, i.e., gene therapy, and therapy with ceasing the expression of the polypeptide by using antisense DNA (RNA). Furthermore, a genomic DNA can be isolated by using the DNA of the present invention as a probe. Similarly, a human gene for a related polypeptide being highly homologous with the DNA of the present invention and a gene of an organism other than human for a polypeptide being high homologous with the polypeptide of the present invention can be isolated.

Examples

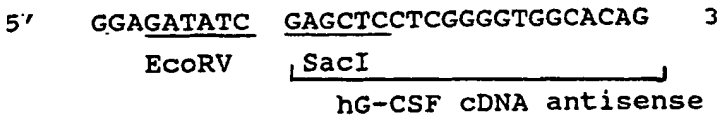
[0041] The following Examples and Reference Example are illustrated, but not limit the present invention.

Reference Example 1

[0042] Construction and expression of plasmid pcDL-SRα-h-G-CSF-hTac (pSGT) and plasmid pcDL-SRα-hRARα-hTac (pSRT)

[0043] A plasmid, wherein a cDNA coding for a fusion protein of hG-CSF (human granulocyte colony stimulating factor, a typical example of a protein having a signal peptide) or hRARα (human retinoic acid receptor α, a typical example of a protein having no signal peptide), with hTac (human IL-2 receptor α, used as a reporter gene) was integrated into an eucaryotic cell expression plasmid vector pcDL-SRα having an SRα promoter [described in Mol. Cell. Biol., 8, 466 (1988), provided by Dr. Yutaka Takebe, National Institute of Health], was constructed. After transformation, the expression of the reporter protein on the membrane was examined.

(1) By employing a plasmid pSP72-hG-CSF, wherein hG-CSF cDNA had been integrated into the EcoRI site of a plasmid pSP72 (purchased from Promega), as a template and using an SP6 promoter primer (purchased from Takara Shuzo Co., Ltd.) and an hG-CSF specific primer having an SacI site added thereto (SEQ ID No. 5):



PCR was performed 25 cycles (at 95 °C for one minute, at 48 °C for two minutes and at 72 °C for two minutes). The amplified DNA fragment was digested with SacI-EcoRI and once subcloned into a plasmid pBlue script SK(+) (pBS). After digesting with SacI-EcoRI again, an EcoRI-SacI fragment of hG-CSF was obtained. On the other hand, a plasmid pBS-hTac, wherein hTac cDNA had been integrated into the HindIII site of pBS, was digested with SacI-KpnI to thereby give an SacI-KpnI fragment of hTac cDNA with the deletion of the signal sequence. These fragments were integrated into the EcoRI-KpnI site of pcDL-SRα with the deletion of stuffer (Fig. 2) to thereby give

a plasmid pcDL-SRα-hG-CSF-hTac (pSGT) (Figs. 3, 4 and 5).

Next, by employing a plasmid pGEM3-hRARα, wherein hRARα cDNA had been integrated into the EcoRI site of a plasmid pGEM3, as a template and using an SP6 promoter primer and an hRARα specific primer having an SacI site added thereto (SEQ ID No. 6):

5′ GGAGATATC GAGCTCAATGGTGGCTGGGGATG 3′
EcoRV SacI
hRARα cDNA antisense

PCR was performed. Subsequently, the procedure employed in the above-mentioned case of G-CSF was repeated to thereby give a plasmid pcDL-SRα-hRARα-hTac (pSRT) (Figs. 6, 4 and 5).

(2) The pSGT or pSRT obtained in the above (1) was transfected into COS-7 cells by the DEAE-dextran method [described in detail in Current Protocol in Molecular Biology, chapter 9.2.1.]. After 48 hours, the cells were harvested from a dish and incubated together with mouse anti-Tac IgG antibody for 20 minutes on ice. After eliminating free antibodies, the mixture was incubated together with goat anti-mouse IgG antibody labeled with fluorescein isothiocyanate (FITC) for 20 minutes on ice. After eliminating free antibodies again, the expression of a fusion protein G-CSF-Tac or RARα-Tac on the membrane was examined with a fluorescence activated cell sorter (Model FACS Can, manufactured by BECTON DICKINSON, hereinafter referred to simply as FACS). Figs. 7 and 8 show the results of the judgement.

[0044] As shown in Fig. 7, G-CSF-Tac was expressed on the membrane as well as Tac. As shown in Fig. 8, on the other hand, RARα-Tac was not detected on the membrane but remained within the cells.

Example 1

Construction of cDNA library having selectivity for signal peptides (Figs. 9 and 10)

[0045] Total RNA was extracted from a mouse stroma cell line ST2 [cells supporting the survival and proliferation of hematopoietic stem cells and the proliferation and differentiation of B cells and myeloid cells; refer to EMBO J., 7, 1337 (1988)] by the acid guanidine-phenol-chloroform (AGPC) method [described in detail in "Saibo Kogaku Jikken Protokoru (Protocol in Cellular Engineering Experiments)", published by Shujun-sha, 28 - 31]. Then poly A-RNA was purified by using oligo (dT)-latex (Oligotex-dT30®, marketed from Takara Shuzo Co., Ltd.). By using a random hexamer as a primer, a single-stranded cDNA was synthesized with reverse transcriptase and dT was added to the 3'-end thereof with the use of terminal deoxytransferase. A 17 mer dA ligated to a restriction enzyme site containing EcoRI (SEQ ID No. 7):

5′ GATGCGGCCGC CTCGAG GAATTC $(dA)_{17}$ 3′
NotI XhoI EcoRI

was annealed and a double-stranded cDNA was synthesized by using the same as a primer. Then the cDNA was fragmentated by ultrasonication so as to give an average length of 300 bp and the cDNAs of 200 to 500 bp were fractionated by agarose gel electrophoresis. After blunting the ends with T4DNA polymerase, a lone linker containing an SacI site

5′ GAGGTACAAGCTT GATATC GAGCTCGCGG 3′ (SEQ ID No.8)
3′ CATGTTCGAA CTATAG CTCGAGCGCC 5′ (SEQ ID No.9)
Hind III EcoRV SacI

[see Nucleic Acids Res., 18, 4293 (1990)] was ligated and cDNAs of 200 to 500 bp were fractionated again by agarose gel electrophoresis. By using a primer (NLC) containing an EcoRi site (SEQ ID No. 10) :

NLC
5′ GATGCGGCCGCCTCGAGGAATTC 3′

and another primer (LLHES) containing an SacI site (SEQ ID No. 11):

LLHES
5′ GAGGTACAAGCTTGATATCGAGCTCGCGG 3′

PCR was performed 25 cycles (at 94 °C for one minute, at 50 °C for two minutes and at 72 °C for two minutes). The amplified cDNA was digested with SacI and EcoRI and cDNAs of 250 to 500 bp were fractionated by agarose gel electrophoresis. The cDNA was ligated to a plasmid obtained by digesting pSRT (prepared in Reference Example 1) with SacI and EcoRI by using T4 DNA ligase. After transformation of an *E. coli* DH5α strain, a cDNA library having a selectivity for signal peptides was obtained.

Example 2

Screening and analysis of cDNA coding for signal peptide

[0046] About 1,200 colonies in the library obtained in Example 1 were divided into 24 pools (about 50 colonies/pool). Plasmids of each pools were isolated by the miniprep method and transfected into COS-7 cells by the DEAE-dextran method. After 48 to 72 hours, cell surface-staining for Tac of the transfectant was performed in the same manner as described in Reference Example 1 and 6 positive pools were selected under a fluorescent microscope. Colonies of one pool from among the 6 positive pools were further divided and the same procedure as described above was repeated until a single clone was obtained. Thus a positive clone (pS-TT3) was obtained. Subsequently, by using two synthetic primers (SEQ ID No. 12):

5′ TTTACTTCTAGGCCTGTACG 3′

(20 bases upstream from EcoRI cloning site, for sense) and (SEQ ID No. 13):

5′ CCATGGCTTTGAATGTGGCG 3′

(20 bases downstream from SacI cloning site, for antisense) which were specific for the pcDL-SRα-Tac vector, the nucleotide sequence of the TT3 insert was determined. An open reading frame following the Tac cDNA with the deletion of the signal sequence in-frame was searched and converted into the deduced amino acid sequence. After performing

a hydrophobicity profile, it was confirmed that a hydrophobic region characteristic to a signal peptide was contained therein (Fig. 11). Further, the homology with data base on DNA and amino acid levels was examined. As a result, it has been found out that TT3 clone codes for an unknown protein.

Example 3

Screening of cDNA with the full length and determination of nucleotide sequence

[0047] A cDNA library was constructed by using Super Script® Ramda System (marketed from BRL). Next, pS-TT3 was digested with SacI and EcoRI and a TT3 cDNA fragment was prepared by agarose gel electrophoresis. The library was screened by using an oligo-labeled TT3 cDNA fragment as a probe and thus a number of positive clones were obtained. Among these clones, a TT3-1-6 clone showing the longest insert was selected and an SalI-NotI fragment excised from a λgt22A vector was subcloned into pBS to thereby give a plasmid pBS-TT316. By using a T7 primer, the nucleotide sequence of 300 bp in the 5'-terminal of TT3-1-6 cDNA was determined. Thus it was confirmed that the sequence identical with TT3 of the probe existed in the most 5'-end of TT3-1-6.

[0048] Next, a number of pBS-TT316 variant plasmids lacking of the 5'-end or the 3'-end of TT3-1-6 cDNA were constructed by using an ExoIII/Mung Bean Deletion Kit (manufactured by Stratagene). By using these variant plasmids, the nucleotide sequence of the full length cDNA was determined (SEQ ID No. 3). From the full length cDNA sequence data, an open reading frame was determined and further translated into an amino acid sequence. Thus the sequence represented by Seq. ID No. 1 was obtained. The amino acid sequence at the 30- to 40-positions in the N-terminal of the amino acid sequence thus obtained were compared with known signal peptides. Thus the signal peptide part of this polypeptide was deduced to thereby give the sequence represented by Seq. ID No. 1.

SEQUENCE LISTING

[0049]

(1) GENERAL INFORMATION:

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(F) POSTAL CODE (ZIP): 541

(ii) TITLE OF INVENTION: PROCESS FOR CONSTRUCTING A cDNA LIBRARY AND A NOVEL POLYPEPTIDE AND DNA ENCODING THE SAME

(iii) NUMBER OF SEQUENCES: 13

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0. Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA: APPLICATION NUMBER: EP 94300267.5

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1797 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 82..138

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 139..348

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GACCACTTTC CCTCTCGGTC CACCTCGGTG TCCTCTTGCT GTCCAGCTCT GCAGCCTCCG 60

GCGCGCCCTC CCGCCCACGC C ATG GAC GCC AAG GTC GTC GCC GTG CTG GCC 111

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                                    Met Asp Ala Lys Val Val Ala Val Leu Ala
                                                   -15                 -10

CTG GTG CTG GCC GCG CTC TGC ATC AGT GAC GGT AAA CCA GTC AGC CTG                159
Leu Val Leu Ala Ala Leu Cys Ile Ser Asp Gly Lys Pro Val Ser Leu
                -5                   1                   5

AGC TAC CGA TGC CCC TGC CGG TTC TTC GAG AGC CAC ATC GCC AGA GCC                207
Ser Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser His Ile Ala Arg Ala
        10                  15                  20

AAC GTC AAG CAT CTG AAA ATC CTC AAC ACT CCA AAC TGT GCC CTT CAG                255
Asn Val Lys His Leu Lys Ile Leu Asn Thr Pro Asn Cys Ala Leu Gln
    25                  30                  35

ATT GTT GCA CGG CTG AAG AAC AAC AAC AGA CAA GTG TGC ATT GAC CCG                303
Ile Val Ala Arg Leu Lys Asn Asn Asn Arg Gln Val Cys Ile Asp Pro
 40                  45                  50                  55

AAA TTA AAG TGG ATC CAA GAG TAC CTG GAG AAA GCT TTA AAC AAG                    348
Lys Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys Ala Leu Asn Lys
                60                  65                  70

TAAGCACAAC AGCCCAAAGG ACTTTCCAGT AGACCCCCGA GGAAGGCTGA CATCCGTGGG              408

AGATGCAAGG GCAGTGGTGG GGAGGAGGGC CTGAACCCTG GCCAGGATGG CCGGCGGGAC              468

AGCACTGACT GGGGTCATGC TAAGGTTTGC CAGCATAAAG ACACTCCGCC ATAGCATATG              528

GTACGATATT GCAGCTTATA TTCATCCCTG CCCTCGCCCG TGCACAATGG AGCTTTTATA              588

ACTGGGGTTT TTCTAAGGAA TTGTATTACC CTAACCAGTT AGCTTCATCC CCATTCTCCT              648

CATCCTCATC TTCATTTTAA AAAGCAGTGA TTACTTCAAG GGCTGTATTC AGTTTGCTTT              708

GGAGCTTCTC TTTGCCCTGG GGCCTCTGGG CACAGTTATA GACGGTGGCT TTGCAGGGAG              768

CCCTAGAGAG AAACCTTCCA CCAGAGCAGA GTCCGAGGAA CGCTGCAGGG CTTGTCCTGC              828

AGGGGGCGCT CCTCGACAGA TGCCTTGTCC TGAGTCAACA CAAGATCCGG CAGAGGGAGG              888

CTCCTTTATC CAGTTCAGTG CCAGGGTCGG GAAGCTTCCT TTAGAAGTGA TCCCTGAAGC              948

TGTGCTCAGA GACCCTTTCC TAGCCGTTCC TGCTCTCTGC TTGCCTCCAA ACGCATGCTT             1008

CATCTGACTT CCGCTTCTCA CCTCTGTAGC CTGACGGACC AATGCTGCAA TGGAAGGGAG             1068

GAGAGTGATG TGGGGTGCCC CCTCCCTCTC TTCCCTTTGC TTTCCTCTCA CTTGGGCCCT             1128

TTGTGAGATT TTTCTTTGGC CTCCTGTAGA ATGGAGCCAG ACCATCCTGG ATAATGTGAG             1188

AACATGCCTA GATTTACCCA CAAAACACAA GTCTGAGAAT TAATCATAAA CGGAAGTTTA             1248

AATGAGGATT TGGACCTTGG TAATTGTCCC TGAGTCCTAT ATATTTCAAC AGTGGCTCTA             1308

TGGGCTCTGA TCGAATATCA GTGATGAAAA TAATAATAAT AATAATAATA ACGAATAAGC             1368

CAGAATCTTG CCATGAAGCC ACAGTGGGGA TTCTGGGTTC CAATCAGAAA TGGAGACAAG             1428
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ATAAAACTTG CATACATTCT TATGATCACA GACGGCCCTG GTGGTTTTTG GTAACTATTT   1488
ACAAGGCATT TTTTTACATA TATTTTTGTG CACTTTTTAT GTTTCTTTGG AAGACAAATG   1548
TATTTCAGAA TATATTTGTA GTCAATTCAT ATATTTGAAG TGGAGCCATA GTAATGCCAG   1608
TAGATATCTC TATGATCTTG AGCTACTGGC AACTTGTAAA GAAATATATA TGACATATAA   1668
ATGTATTGTA GCTTTCCGGT GTCAGCCACG GTGTATTTTT CCACTTGGAA TGAAATTGTA   1728
TCAACTGTGA CATTATATGC ACTAGCAATA AAATGCTAAT TGTTTCATGC TGTAAAAAAA   1788
AAAAAAAAA                                                           1797
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Asp Ala Lys Val Val Ala Val Leu Ala Leu Val Leu Ala Ala Leu
 1               5                  10                  15

Cys Ile Ser Asp Gly Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys
            20                  25                  30

Arg Phe Phe Glu Ser His Ile Ala Arg Ala Asn Val Lys His Leu Lys
        35                  40                  45

Ile Leu Asn Thr Pro Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys
    50                  55                  60

Asn Asn Asn Arg Gln Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln
65                  70                  75                  80

Glu Tyr Leu Glu Lys Ala Leu Asn Lys
                85
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1797 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACCACTTTC CCTCTCGGTC CACCTCGGTG TCCTCTTGCT GTCCAGCTCT GCAGCCTCCG 60

GCGCGCCCTC CCGCCCACGC CATGGACGCC AAGGTCGTCG CCGTGCTGGC CCTGGTGCTG 120

GCCGCGCTCT GCATCAGTGA CGGTAAACCA GTCAGCCTGA GCTACCGATG CCCCTGCCGG 180

TTCTTCGAGA GCCACATCGC CAGAGCCAAC GTCAAGCATC TGAAAATCCT CAACACTCCA 240

AACTGTGCCC TTCAGATTGT TGCACGGCTG AAGAACAACA ACAGACAAGT GTGCATTGAC 300

CCGAAATTAA AGTGGATCCA AGAGTACCTG GAGAAAGCTT TAAACAAGTA AGCACAACAG 360

CCCAAAGGAC TTTCCAGTAG ACCCCCGAGG AAGGCTGACA TCCGTGGGAG ATGCAAGGGC 420

AGTGGTGGGG AGGAGGGCCT GAACCCTGGC CAGGATGGCC GGCGGGACAG CACTGACTGG 480

GGTCATGCTA AGGTTTGCCA GCATAAAGAC ACTCCGCCAT AGCATATGGT ACGATATTGC 540

AGCTTATATT CATCCCTGCC CTCGCCCGTG CACAATGGAG CTTTTATAAC TGGGGTTTTT 600

CTAAGGAATT GTATTACCCT AACCAGTTAG CTTCATCCCC ATTCTCCTCA TCCTCATCTT 660

CATTTTAAAA AGCAGTGATT ACTTCAAGGG CTGTATTCAG TTTGCTTTGG AGCTTCTCTT 720

TGCCCTGGGG CCTCTGGGCA CAGTTATAGA CGGTGGCTTT GCAGGGAGCC CTAGAGAGAA 780

ACCTTCCACC AGAGCAGAGT CCGAGGAACG CTGCAGGGCT TGTCCTGCAG GGGGCGCTCC 840

TCGACAGATG CCTTGTCCTG AGTCAACACA AGATCCGGCA GAGGGAGGCT CCTTTATCCA 900

GTTCAGTGCC AGGGTCGGGA AGCTTCCTTT AGAAGTGATC CCTGAAGCTG TGCTCAGAGA 960

CCCTTTCCTA GCCGTTCCTG CTCTCTGCTT GCCTCCAAAC GCATGCTTCA TCTGACTTCC 1020

GCTTCTCACC TCTGTAGCCT GACGGACCAA TGCTGCAATG GAAGGGAGGA GAGTGATGTG 1080

GGGTGCCCCC TCCCTCTCTT CCCTTTGCTT TCCTCTCACT TGGGCCCTTT GTGAGATTTT 1140

TCTTTGGCCT CCTGTAGAAT GGAGCCAGAC CATCCTGGAT AATGTGAGAA CATGCCTAGA 1200

TTTACCCACA AAACACAAGT CTGAGAATTA ATCATAAACG GAAGTTTAAA TGAGGATTTG 1260

GACCTTGGTA ATTGTCCCTG AGTCCTATAT ATTTCAACAG TGGCTCTATG GGCTCTGATC 1320

GAATATCAGT GATGAAAATA ATAATAATAA TAATAATAAC GAATAAGCCA GAATCTTGCC 1380

ATGAAGCCAC AGTGGGGATT CTGGGTTCCA ATCAGAAATG GAGACAAGAT AAAACTTGCA 1440

TACATTCTTA TGATCACAGA CGGCCCTGGT GGTTTTTGGT AACTATTTAC AAGGCATTTT 1500

TTTACATATA TTTTTGTGCA CTTTTTATGT TTCTTTGGAA GACAAATGTA TTTCAGAATA 1560

TATTTGTAGT CAATTCATAT ATTTGAAGTG GAGCCATAGT AATGCCAGTA GATATCTCTA 1620

TGATCTTGAG CTACTGGCAA CTTGTAAAGA AATATATATG ACATATAAAT GTATTGTAGC 1680

TTTCCGGTGT CAGCCACGGT GTATTTTTCC ACTTGGAATG AAATTGTATC AACTGTGACA 1740

TTATATGCAC TAGCAATAAA ATGCTAATTG TTTCATGCTG TAAAAAAAAA AAAAAAA 1797

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 267 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATGGACGCCA AGGTCGTCGC CGTGCTGGCC CTGGTGCTGG CCGCGCTCTG CATCAGTGAC 60

GGTAAACCAG TCAGCCTGAG CTACCGATGC CCCTGCCGGT TCTTCGAGAG CCACATCGCC 120

AGAGCCAACG TCAAGCATCT GAAAATCCTC AACACTCCAA ACTGTGCCCT TCAGATTGTT 180

GCACGGCTGA AGAACAACAA CAGACAAGTG TGCATTGACC CGAAATTAAA GTGGATCCAA 240

GAGTACCTGG AGAAAGCTTT AAACAAG 267

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGAGATATCG AGCTCCTCGG GGTGGCACAG 30

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGAGATATCG AGCTCAATGG TGGCTGGGGA TG 32

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GATGCGGCCG CCTCGAGGAA TTCAAAAAAA AAAAAAAAAA 40

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GAGGTACAAG CTTGATATCG AGCTCGCGG 29

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCGCGAGCTC GATATCAAGC TTGTAC 26

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GATGCGGCCG CCTCGAGGAA TTC 23

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GAGGTACAAG CTTGATATCG AGCTCGCGG 29

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TTTACTTCTA GGCCTGTACG 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCATGGCTTT GAATGTGGCG 20

Claims

1. A polypeptide in substantially purified form having the amino acid sequence shown in SEQ ID NO. 1, the amino acid sequence of residues 1 to 70 shown in SEQ ID NO. 1 or an amino acid sequence having at least 90% homology with the amino acid sequence shown in SEQ ID NO. 1 over its entire length or with the amino acid sequence of residues 1 to 70 shown in SEQ ID NO. 1.

2. A polypeptide according to claim 1 having the amino acid sequence shown in SEQ ID NO. 1.

3. A polypeptide according to claim 1 having the amino acid sequence of residues 1 to 70 shown in SEQ ID NO. 1.

4. DNA encoding a polypeptide according to any one of the preceding claims.

5. DNA according to claim 4 having the nucleotide sequence shown in SEQ ID NO. 4.

6. DNA according to claim 4 having the nucleotide sequence shown in SEQ ID No. 3.

7. DNA according to claim 4 having the nucleotide sequence of residues 139 to 348 shown in SEQ ID NO. 1.

8. A replication and expression vector comprising DNA according to any one of claims 4 to 7.

9. A host cell transformed or transfected with a replication and expression vector according to claim 8.

10. A method of producing a polypeptide which comprises culturing host cells according to claim 10 under conditions effective to express a polypeptide according to any one of claims 1 to 3.

11. A monoclonal or polyclonal antibody to a polypeptide according to any one of claims 1 to 3.

12. A pharmaceutical composition containing a polypeptide according to any one of claims 1 to 3 or an antibody according to claim 11 in association with a pharmaceutically acceptable diluent or carrier.

Patentansprüche

1. Polypeptid in im wesentlichen gereinigter Form mit der Aminosäuresequenz, die in SEQ ID NO. 1 angegeben ist, mit der Aminosäuresequenz der Reste 1 bis 70, die in SEQ ID NO. 1 angegeben ist, oder mit einer Aminosäuresequenz mit mindestens 90 % Homologie mit der Aminosäuresequenz, die in SEQ ID NO. 1 angegeben ist, über deren gesamte Länge oder mit der Aminosäuresequenz der Reste 1 bis 70, die in SEQ ID NO. 1 angegeben ist.

2. Polypeptid nach Anspruch 1 mit der Aminosäuresequenz, die in SEQ ID NO. 1 angegeben ist.

3. Polypeptid nach Anspruch 1 mit der Aminosäureseqüenz der Reste 1 bis 70, die in SEQ ID NO. 1 angegeben ist.

4. DNA mit Kodierung für ein Polypeptid gemäß einem der vorhergehenden Ansprüche.

5. DNA nach Anspruch 4 mit der Nucleotidsequenz, die in SEQ ID NO. 4 angegeben ist.

6. DNA nach Anspruch 4 mit der Nucleotidsequenz, die in SEQ ID NO. 3 angegeben ist.

7. DNA nach Anspruch 4 mit der Nucleotidsequenz der Reste 139 bis 348, die in SEQ ID NO. 1 angegeben ist.

8. Replikations- und Expressionsvektor, der DNA nach einem der Ansprüche 4 bis 7 umfasst.

9. Wirtszelle, die mit einem Replikations- und Expressionsvektor nach Anspruch 8 transformiert oder transfiziert wurde.

10. Verfahren zur Herstellung eines Polypeptids, das ein Kultivieren von Wirtszellen nach Anspruch 9 unter Bedingungen, die für eine Expression eines Polypeptids nach einem der Ansprüche 1 bis 3 wirksam sind, umfasst.

11. Monoklonaler oder polyklonaler Antikörper gegen ein Polypeptid nach einem der Ansprüche 1 bis 3.

12. Pharmazeutische Zusammensetzung, die ein Polypeptid nach einem der Ansprüche 1 bis 3 oder einen Antikörper nach Anspruch 11 in Verbindung mit einem pharmazeutisch akzeptablen Verdünnungsmittel oder Träger enthält.

Revendications

1. Polypeptide sous forme essentiellement purifiée, ayant la séquence d'aminoacides présentée dans SEQ ID N° 1, la séquence d'aminoacides des résidus 1 à 70 présentés dans SEQ ID N° 1 ou une séquence d'aminoacides ayant une homologie d'au moins 90 % avec la séquence d'aminoacides présentée dans SEQ ID N° 1 sur toute sa longueur ou avec la séquence d'aminoacides des résidus 1 à 70 présentés dans SEQ ID N° 1.

2. Polypeptide selon la revendication 1, ayant la séquence d'aminoacides présentée dans SEQ ID N° 1.

3. Polypeptide selon la revendication 1, ayant la séquence d'aminoacides des résidus 1 à 70 présentés dans SEQ ID N° 1.

4. ADN codant pour un polypeptide selon l'une quelconque des revendications précédentes.

5. ADN selon la revendication 4, ayant la séquence de nucléotides présentée dans SEQ ID N° 4.

6. ADN selon la revendication 4, ayant la séquence de nucléotides présentée dans SEQ ID N° 3.

7. ADN selon la revendication 4, ayant la séquence de nucléotides des résidus 139 à 348 présentés dans SEQ ID N° 1.

8. Vecteur de réplication et d'expression comprenant un ADN selon l'une quelconque des revendications 4 à 7.

9. Cellule hôte transformée ou transfectée avec un vecteur de réplication et d'expression selon la revendication 8.

10. Procédé de production d'un polypeptide, comprenant la culture de cellules hôtes selon la revendication 9 dans des conditions efficaces pour l'expression d'un polypeptide selon l'une quelconque des revendications 1 à 3.

11. Anticorps monoclonal ou polyclonal dirigé contre un polypeptide selon l'une quelconque des revendications 1 à 3.

12. Composition pharmaceutique contenant un polypeptide selon l'une quelconque des revendications 1 à 3 ou un anticorps selon la revendication 11 en association avec un diluant ou un support pharmaceutiquement acceptable.

Figure 1

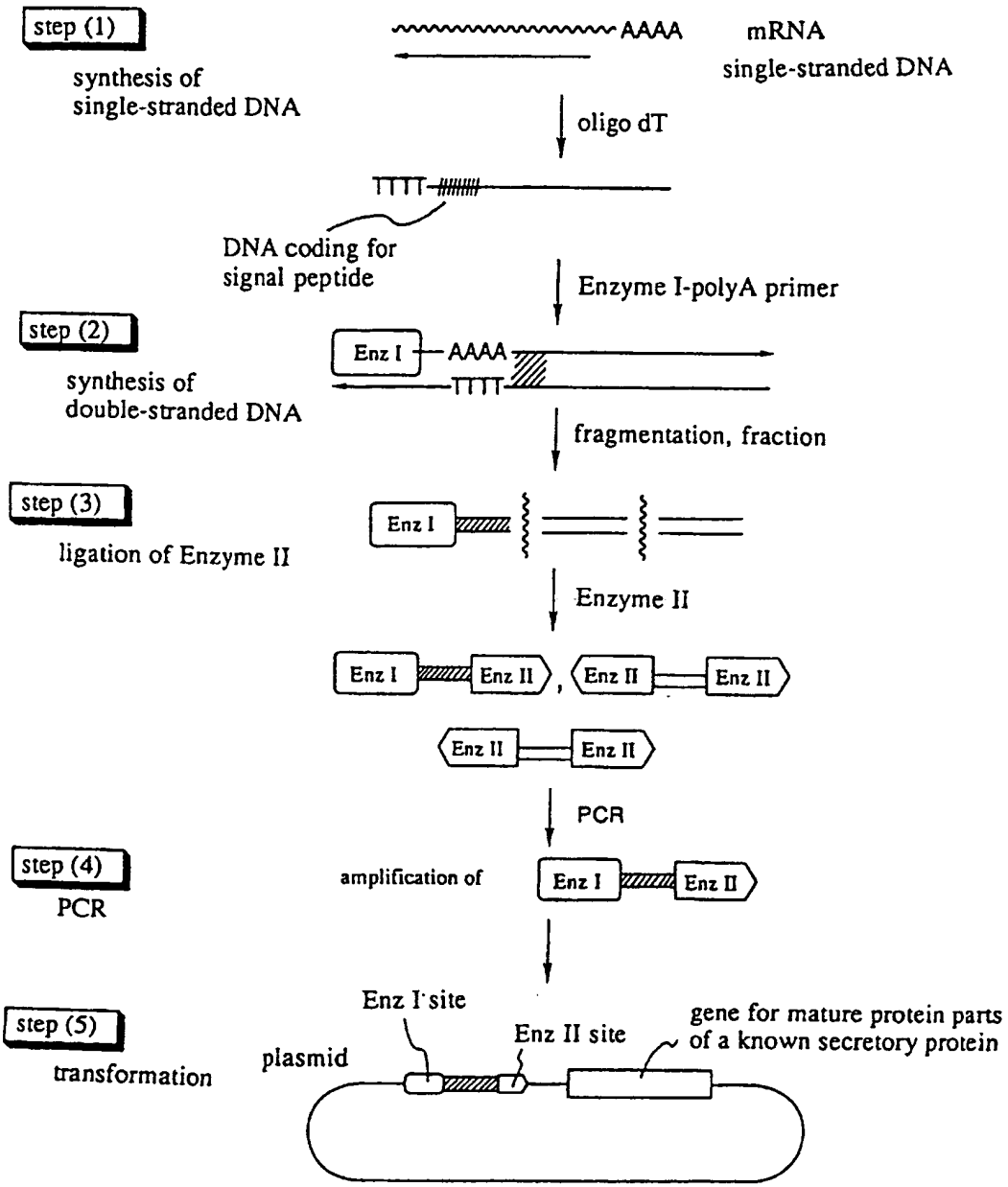

step (1)
synthesis of single-stranded DNA
AAAA
mRNA
single-stranded DNA
oligo dT
TTTT
DNA coding for signal peptide
Enzyme I-polyA primer
step (2)
synthesis of double-stranded DNA
Enz I
AAAA
TTTT
fragmentation, fraction
step (3)
ligation of Enzyme II
Enz I
Enzyme II
Enz I
Enz II
Enz II
Enz II
Enz II
Enz II
PCR
step (4)
PCR
amplification of
Enz I
Enz II
Enz I site
Enz II site
gene for mature protein parts of a known secretory protein
step (5)
transformation
plasmid

Figure 2

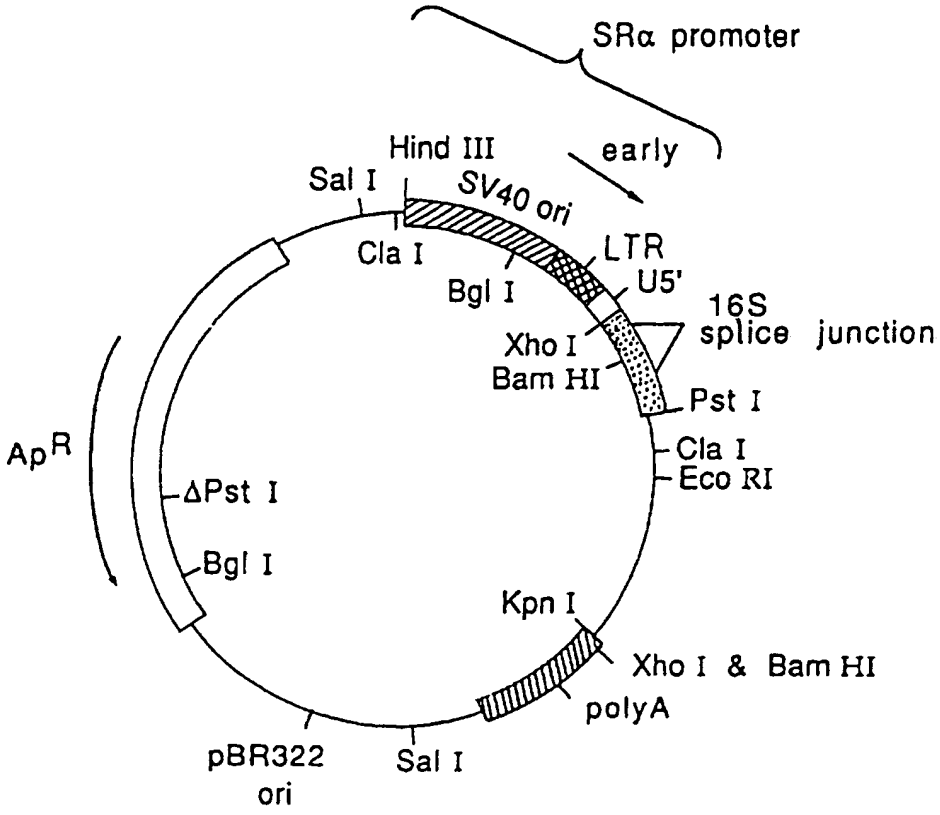

SRα promoter
Hind III
early
Sal I
SV40 ori
Cla I
LTR
Bgl I
U5'
16S
splice junction
Xho I
Bam HI
Pst I
Cla I
Eco RI
ApR
ΔPst I
Bgl I
Kpn I
Xho I & Bam HI
polyA
pBR322
ori
Sal I

Figure 3

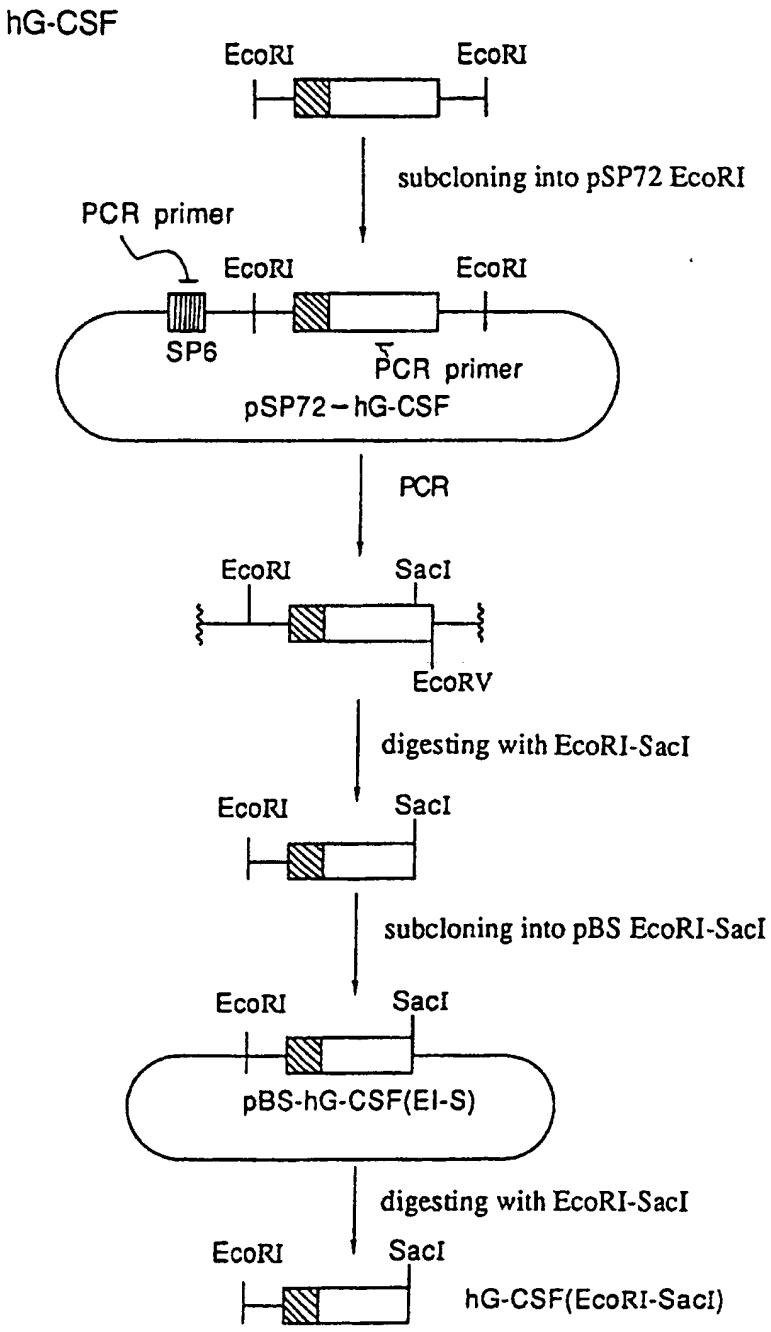

hG-CSF
EcoRI
EcoRI
subcloning into pSP72 EcoRI
PCR primer
EcoRI
EcoRI
SP6
PCR primer
pSP72−hG-CSF
PCR
EcoRI
SacI
EcoRV
digesting with EcoRI-SacI
EcoRI
SacI
subcloning into pBS EcoRI-SacI
EcoRI
SacI
pBS-hG-CSF(EI-S)
digesting with EcoRI-SacI
EcoRI
SacI
hG-CSF(EcoRI-SacI)

Figure 4

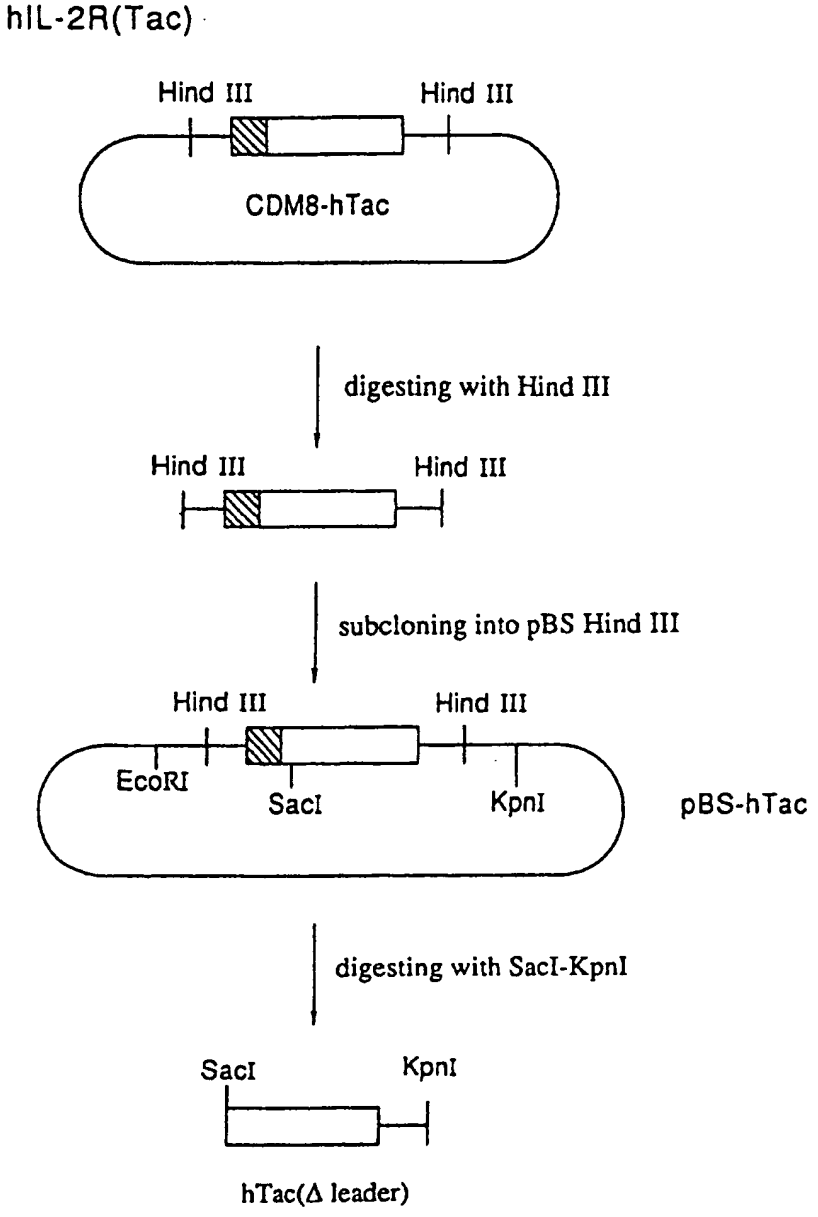
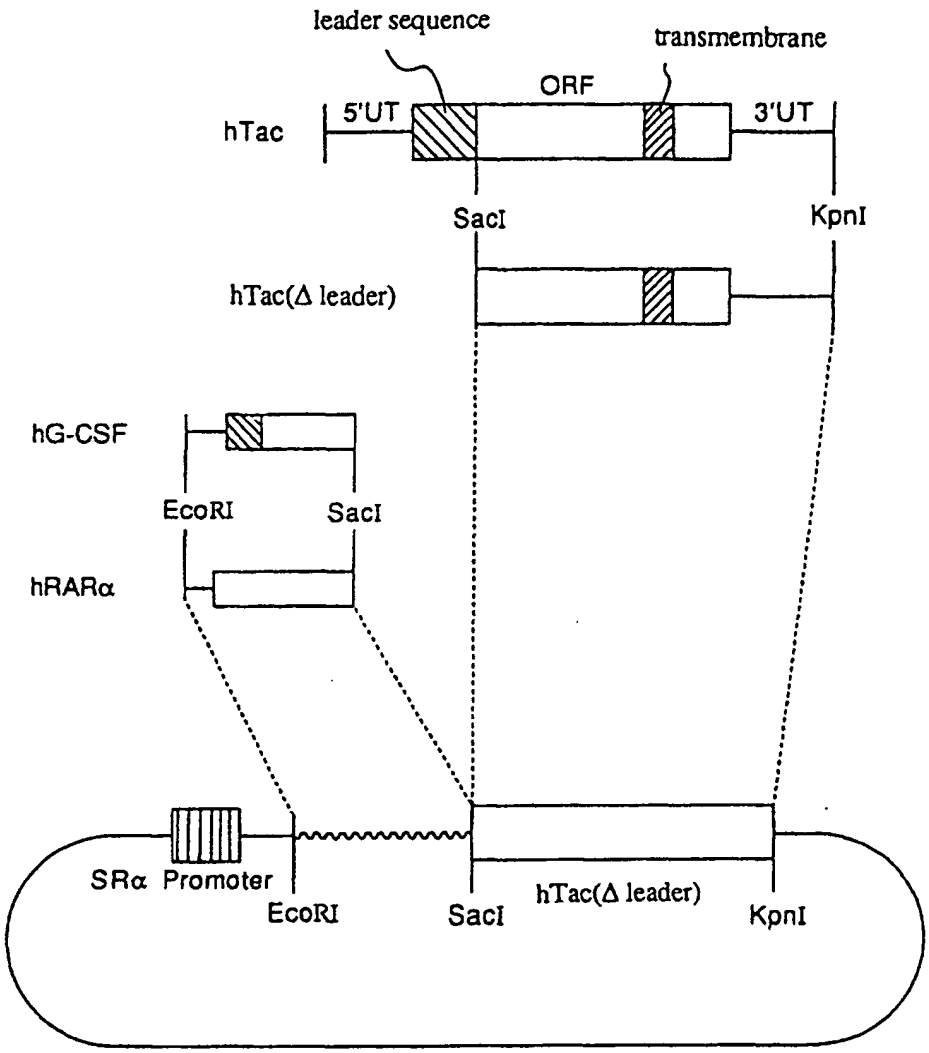

hIL-2R(Tac)
Hind III
Hind III
CDM8-hTac
digesting with Hind III
Hind III
Hind III
subcloning into pBS Hind III
Hind III
Hind III
EcoRI
SacI
KpnI
pBS-hTac
digesting with SacI-KpnI
SacI
KpnI
hTac(Δ leader)

Figure 5



pcDL-SRα-hG-CSF-hTac(Δ leader) (pSGT)

pcDL-SRα-hRARα-hTac(Δ leader) (pSRT)

Figure 6

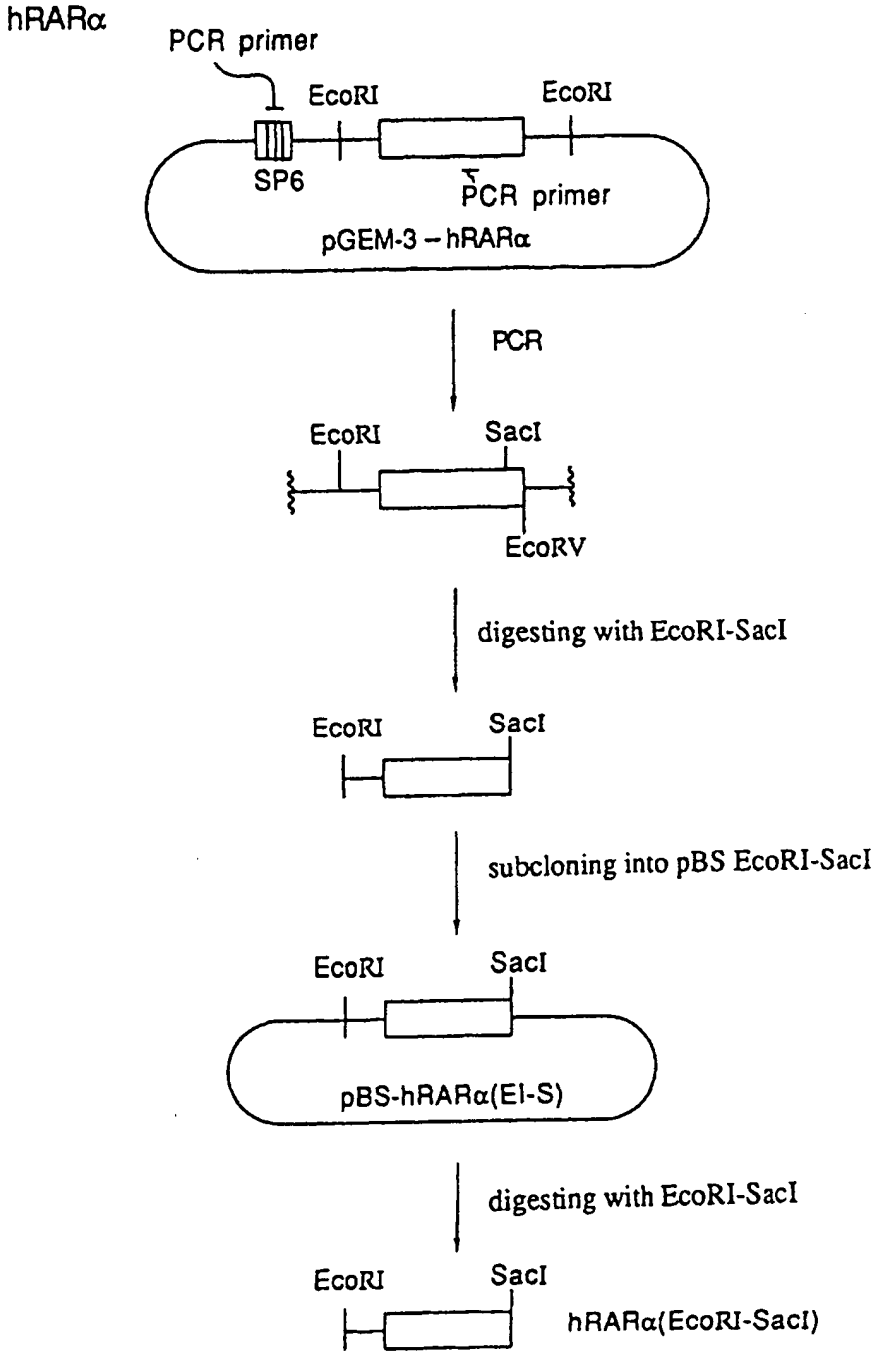

hRARα
PCR primer
EcoRI
EcoRI
SP6
PCR primer
pGEM-3 – hRARα
PCR
EcoRI
SacI
EcoRV
digesting with EcoRI-SacI
EcoRI
SacI
subcloning into pBS EcoRI-SacI
EcoRI
SacI
pBS-hRARα(EI-S)
digesting with EcoRI-SacI
EcoRI
SacI
hRARα(EcoRI-SacI)

Figure 7

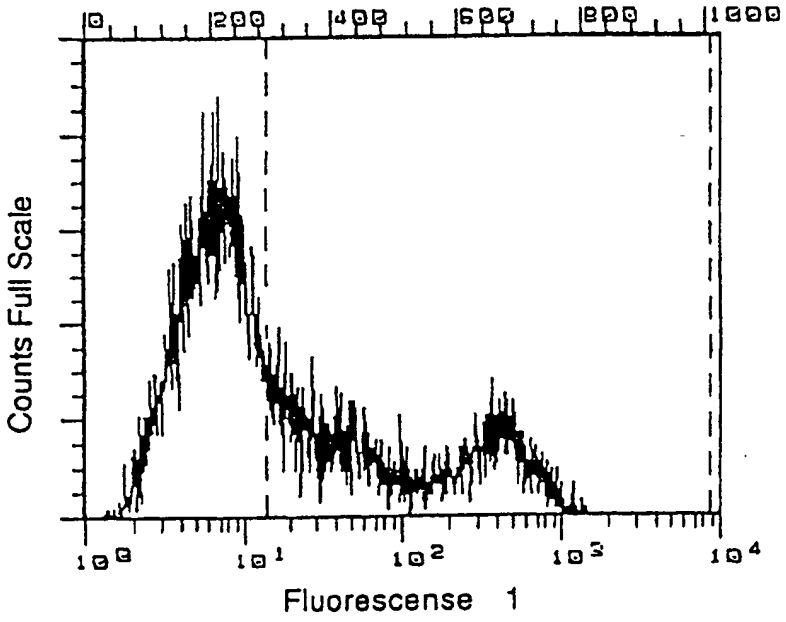

0
200
400
600
800
1000
Counts Full Scale
10^0
10^1
10^2
10^3
10^4
Fluorescense 1

Figure 8

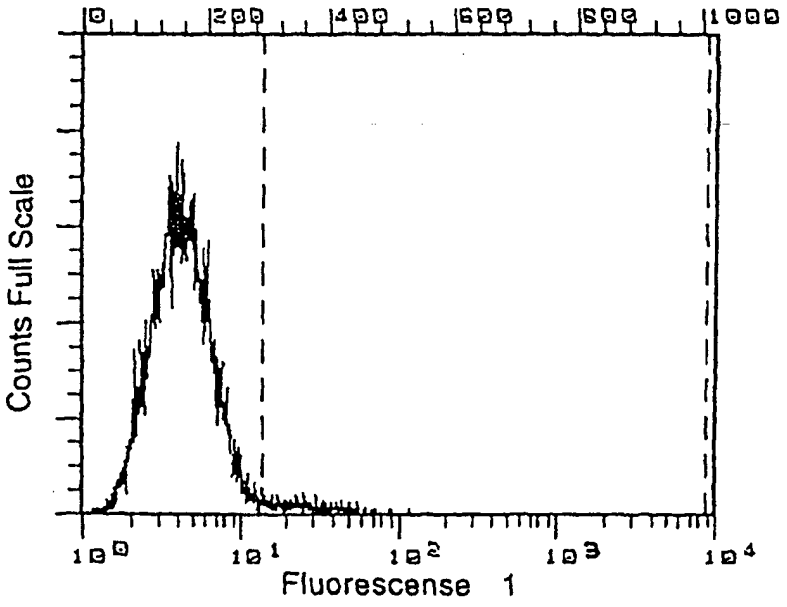

0
200
400
600
800
1000
Counts Full Scale
10^0
10^1
10^2
10^3
10^4
Fluorescense 1

Figure 9

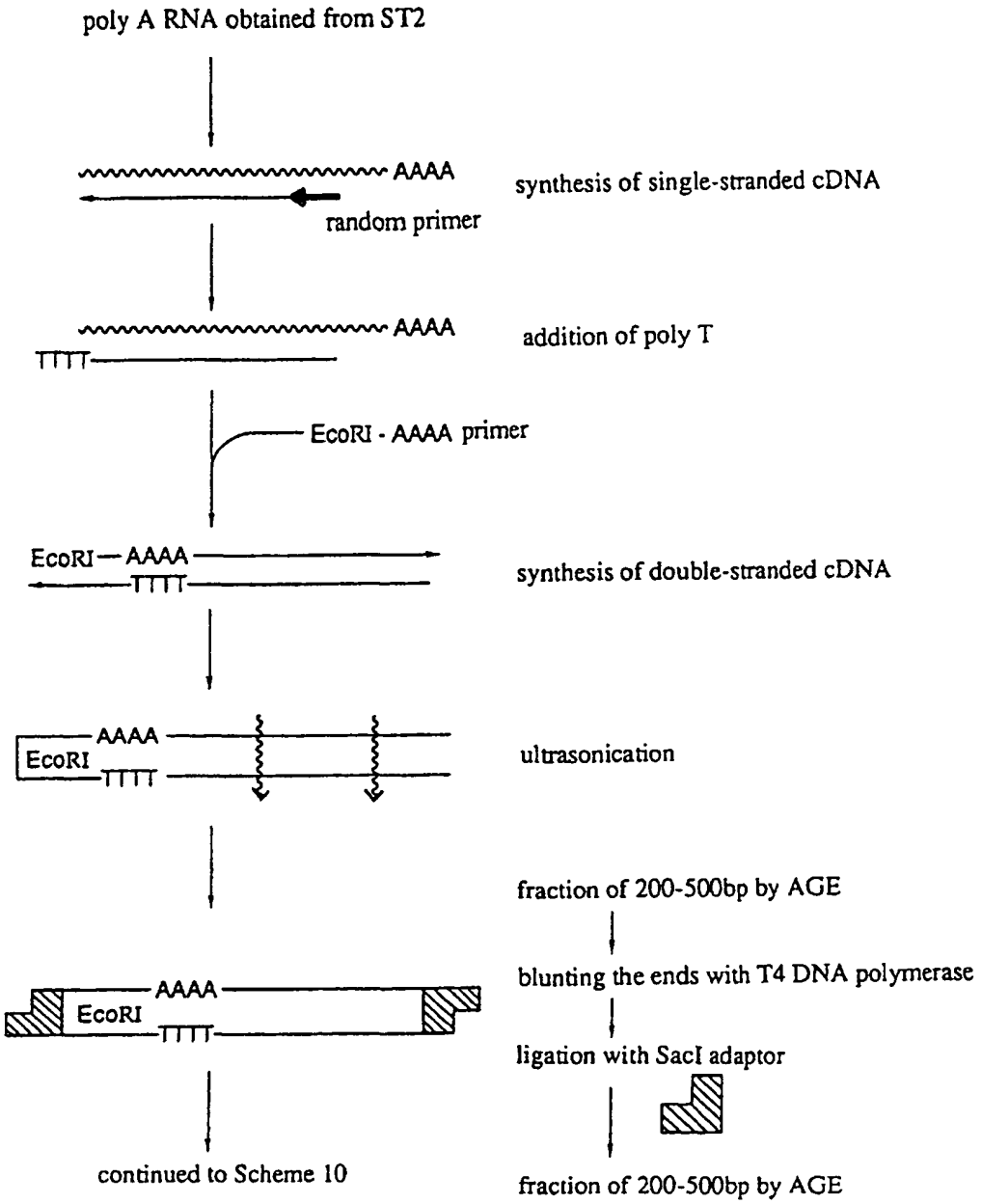

poly A RNA obtained from ST2
AAAA
random primer
synthesis of single-stranded cDNA
AAAA
TTTT
addition of poly T
EcoRI - AAAA primer
EcoRI
AAAA
TTTT
synthesis of double-stranded cDNA
AAAA
EcoRI
TTTT
ultrasonication
fraction of 200-500bp by AGE
blunting the ends with T4 DNA polymerase
AAAA
EcoRI
TTTT
ligation with SacI adaptor
continued to Scheme 10
fraction of 200-500bp by AGE

Figure 10

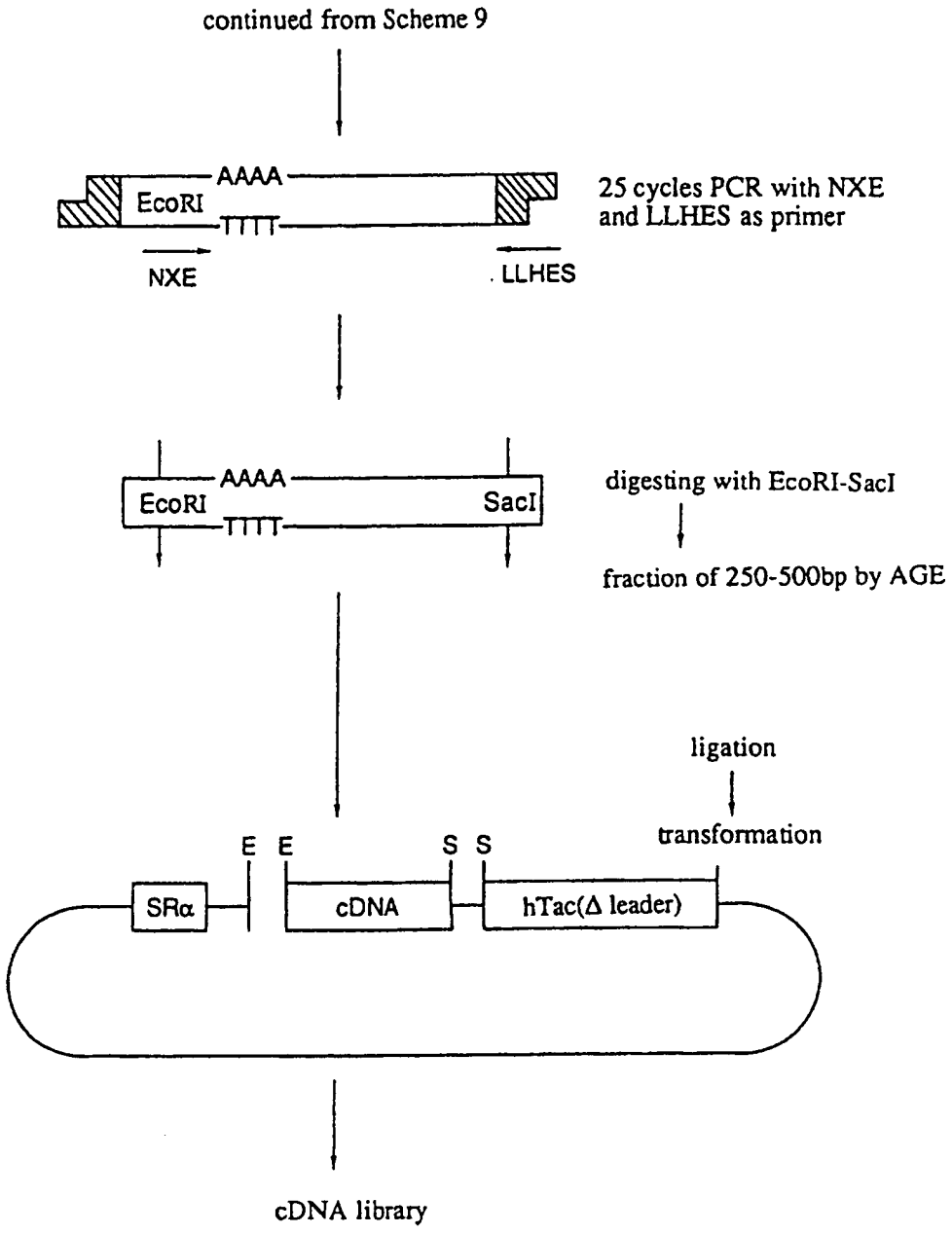

continued from Scheme 9
AAAA
EcoRI
TTTT
NXE
LLHES
25 cycles PCR with NXE and LLHES as primer
AAAA
EcoRI
SacI
TTTT
digesting with EcoRI-SacI
fraction of 250-500bp by AGE
ligation
transformation
E E
S S
SRα
cDNA
hTac(Δ leader)
cDNA library

Figure 11